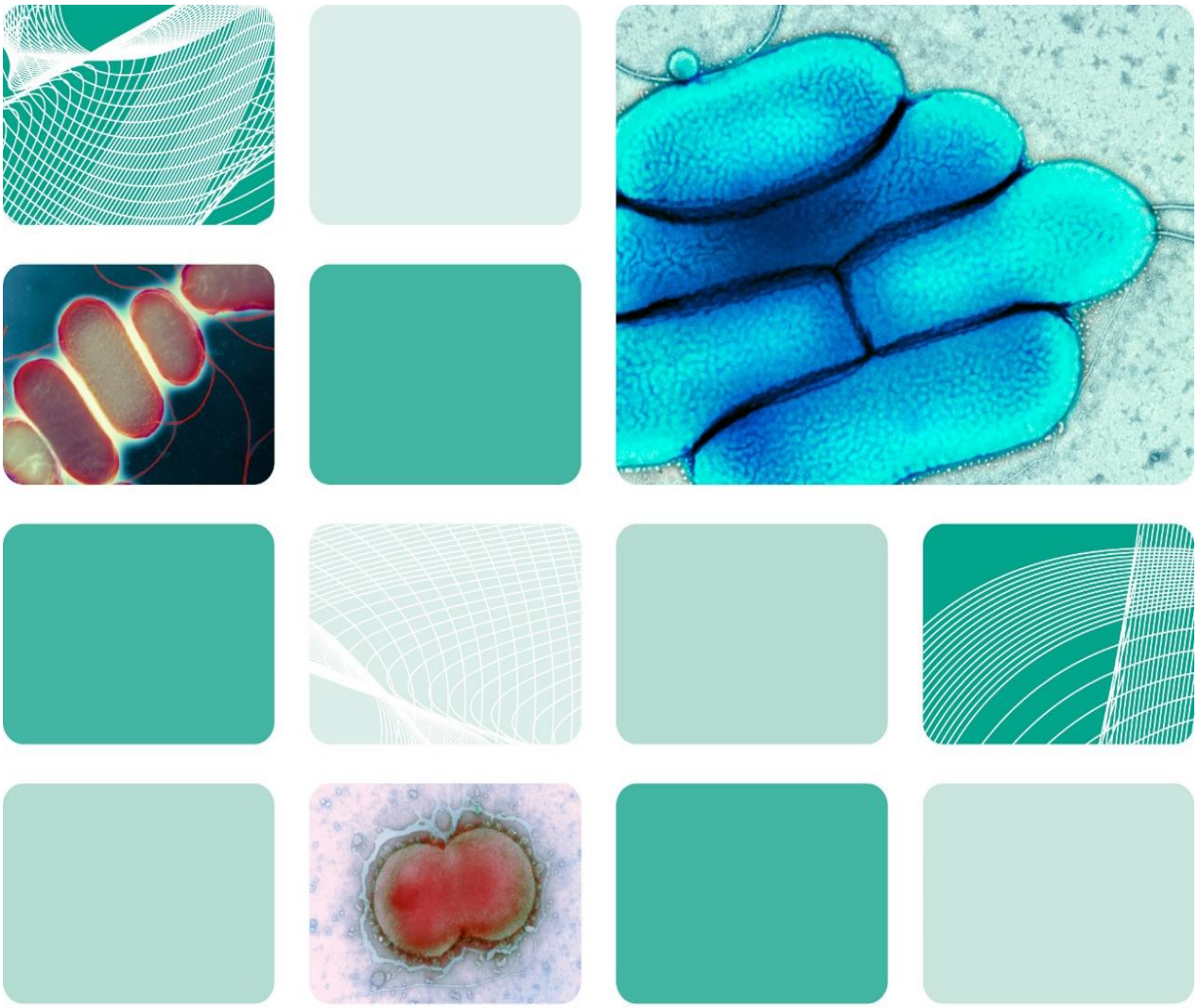
UK Health Security Agency

UK Standards for Microbiology Investigations

Catalase test



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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	8/18.02.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 02.04.2019.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

Amendment number/date	7/02.04.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	02.04.22
Section(s) involved	**Amendment**
Whole document.	Document and flowchart updated.

	Technical limitations updated with subheadings. References updated with grades. Alternative positive bacterial NCTC strain (NCTC 12973) tested and validated for this test and EUCAST susceptibility tests. Fungal NCPF strains added.

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This test detects the catalase enzyme present in most cytochrome-containing aerobic and facultative anaerobic bacteria[1]. *Streptococcus* and *Enterococcus* species are exceptions. Yeast such as *Cryptococcus neoformans* is catalase positive and can be presumptively identified using catalase test[2].

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

The catalase test is used to detect the presence of catalase enzyme by the decomposition of hydrogen peroxide to release oxygen and water as shown by the following reaction:

$$2\ H_2O_2 \rightarrow 2H_2O + O_2$$

The catalase reaction is evident by the rapid formation of bubbles.

Hydrogen peroxide is formed by some bacteria as an oxidative end product of the aerobic breakdown of sugars. If allowed to accumulate, it is highly toxic to bacteria and can result in cell death. Catalase either decomposes hydrogen peroxide or oxidises secondary substrates, but it has no effect on other peroxides[2].

There are method variations of the catalase test and these include the slide test method, the tube or bottle method and the agar slant method[3]. However, the commonly used methods in microbiology laboratories are the tube or bottle method and the agar slant method because it limits catalase aerosols, which have been shown to carry viable bacterial cells, that if inhaled could cause infections as well as contamination in other laboratory work being set up and work surface areas[4].

5 Technical information/limitations

5.1 Interpretation of results

Media containing whole red blood cells will contain catalase and could therefore give a false positive result.

The enzyme, catalase is present in viable cultures only, so colony growth must be from an 18 to 24hr culture. Older cultures may lose their catalase activity and give false negative reactions[2].

Some inoculating loops or wires (nichrome) can react with the hydrogen peroxide to produce false positive reactions[5].

False positive results can also be produced by dirty glass test tubes or bijoux bottles[6].

5.2 False reactions

A weak catalase or pseudocatalase reaction may be produced by some strains of *Aerococcus* species. Some strains of *Enterococcus* species also produce a pseudocatalase.

Cultures of anaerobic bacteria should be exposed to air for 30 min prior to testing[2].

5.3 Quality control

Hydrogen peroxide is unstable and must be refrigerated at all times. Avoid any undue exposure to light.

6 Safety considerations[7-24]

Refer to current guidance on the safe handling of all organisms and reagents documented in this UK SMI.

Catalase testing of bacteria can be hazardous due to the release of bacteria-laden aerosols by liberated oxygen[4]. All work likely to generate aerosols must be performed in a microbiological safety cabinet.

Hydrogen peroxide is a highly corrosive chemical (depending on the concentration); therefore, appropriate personal protective clothing must be worn at all times when in use. Extreme care must be taken by persons using this reagent.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

7 Reagents and equipment[2]

Discrete bacterial/yeast colonies on solid medium

Note: The catalase test should not be performed on colonies taken from media containing whole red blood cells because they contain catalase and could therefore give a false positive result. Colonies taken from chocolate agar plate may be tested as the blood cells have been destroyed[2].

Inoculated pure agar slant culture

Hydrogen peroxide solution, 3–6 %. Commercial preparations are available.

Clean capped test tubes (plastic or glass) or Bijoux bottles

Bacteriological straight platinum wire/loop or disposable alternative

8 Quality control organisms

Bacteria

Positive control:

Staphylococcus aureus NCTC 6571 or NCTC 12973

Negative control:

Streptococcus mitis NCTC 10712

Fungi

Positive control:

Cryptococcus neoformans NCPF 3168

Negative control:

Candida albicans NCPF 3281

Note 1: Hydrogen peroxide is unstable and so should undergo a quality control check daily or immediately prior to use. The positive and negative controls should be run simultaneously.

Note 2: These bacterial strains have been validated by NCTC to give this result.

Note 3: The fungal strains have not been validated by NCTC to give this result at the time of publication.

9 Procedure and results

9.1 Tube or bottle method[3]

- Place 4 to 5 drops of hydrogen peroxide solution in a test tube or bijoux bottle
- Carefully pick a colony to be tested with a wire/loop or disposable alternative
- Rub the colony on the inside wall of the bottle just above the surface of the hydrogen peroxide solution
- Cap the tube or bottle and tilt it to allow the hydrogen peroxide solution to cover the colony
- Observe for immediate bubble formation (effervescence)

9.2 Agar slant method[2,6]

- Add 1.0mL of H_2O_2 directly onto an 18 to 24hr heavily inoculated pure culture grown on a nutrient agar slant and replace the cap
- Observe for immediate bubbling (effervescence)

For both methods,

Positive result

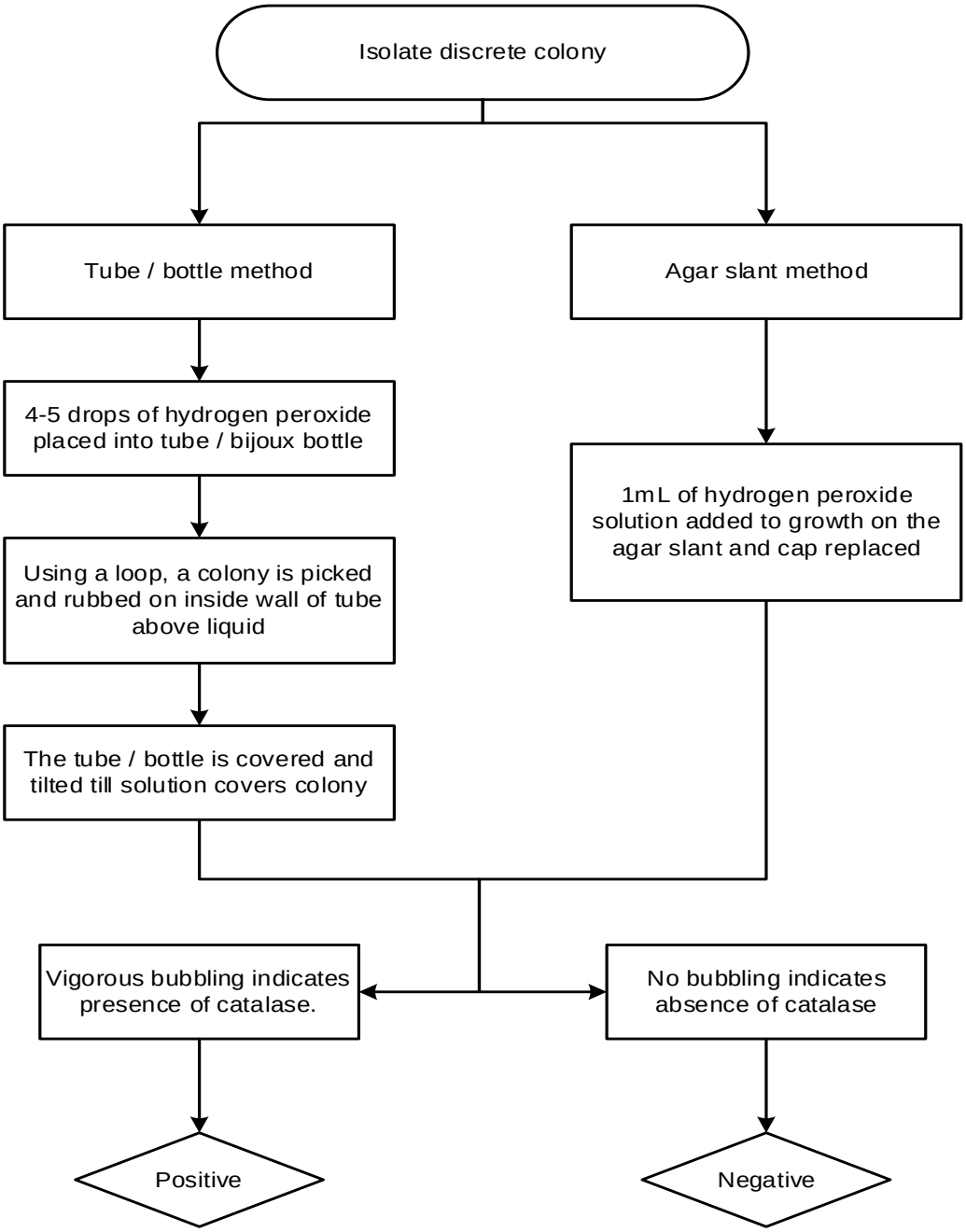
Vigorous bubbling indicates the presence of catalase.

Negative result

No bubbling indicates the absence of catalase.

Note: Both positive and negative controls must be tested alongside the test organism.

Algorithm: Catalase test



Note:

Bacteria
Positive Control: *Staphylococcus aureus* NCTC 6571 or NCTC 12973
Negative Control: *Streptococcus mitis* NCTC 10712

Fungi
Positive control: *Cryptococcus neoformans* NCPF 3168
Negative control: *Candida albicans* NCPF 3281

References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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